L2: Entry 15 of 51 File: USPT Jul 24, 2001

DOCUMENT-IDENTIFIER: US 6265387 B1
TITLE: Process of delivering naked DNA into a hepatocyte via bile duct

Brief Summary Text (6):
Foreign gene expression has also been achieved by repetitively injecting naked DNA in isotonic solutions into the liver parenchyma of animals treated with dexamethasone [(Malone, R. W. et al. JBC 269:29903-29907 (1994) (Hickman, M. A. Human Gene Therapy 5:1477-1483 (1994)]. Plasmid DNA expression in the liver has also been achieved via liposomes delivered by tail vein or intraportal routes [(Kaneda, Y., Kunimitsu, I. and Uchida, T. J. Biol. Chem. 264:12126-12129 (1989) (Soriano, P. et al. PNAS 80:7128-7131 (1983) Kaneda, Y., Iwai, K. and Uchida, T. Science 243:375-378 (1989)].

Brief Summary Text (14):
The term, naked polynucleotides, indicates that the polynucleotides are not associated with a transfection reagent or other delivery vehicle that is required for the polynucleotide to be delivered to the parenchymal cell. A transfection reagent is a compound or compounds used in the prior art that bind(s) to or complex(es) with polynucleotides and mediates their entry into cells. The transfection reagent also mediates the binding and internalization of polynucleotides into cells. Examples of transfection reagents include cationic liposomes and lipids, calcium phosphate precipitates, and polylysine complexes. Typically, the transfection reagent has a net positive charge that binds to the polynucleotide's negative charge. The transfection reagent mediates binding of polynucleotides to cell via its positive charge (that binds to the cell membrane's negative charge) or via ligands that bind to receptors in the cell. For example, cationic liposomes or polylysine complexes have net positive charges that enable them to bind to DNA. Other vehicles are also used, in the prior art, to transfer genes into cells. These include complexing the polynucleotides on particles that are then accelerated into the cell. This is termed biolistic or gun techniques. Other methods include eletroporation in which a device is used to give an electric charge to cells. The charge increases the permeability of the cell.

Brief Summary Text (43):
In another preferred embodiment, the intravascular pressure of a blood vessel is increased by increasing the osmotic pressure within the blood vessel. Typically, hypertonic solutions containing salts such as NaCl, sugars or polyols such as mannitol are used. Hypertonic means that the osmolality of the injection solution is greater than physiologic osmolality. Isotonic means that the osmolality of the injection solution is the same as the physiological osmolality (the tonicity or osmotic pressure of the solution is similar to that of blood). Hypertonic solutions have increased tonicity and osmotic pressure similar to the osmotic pressure of blood and cause cells to shrink.

Other Reference Publication (7):
Stephan, D. et al., "A New Cationic Liposome DNA Complex Enhances The Efficiency Of Arterial Gene Transfer In Vivo," Human Gene Therapy 1996; 7:1803-1812.

Other Reference Publication (29):
Soriano P. et al., "Targeted and nontargeted liposomes for in vivo transfer to rat liver cells of a plasmid containing the preproinsulin I gene." Proc. Matl. Acad. Sci. USA; vol. 80, pp. 7128-7131, Dec. 1983.

L2: Entry 10 of 51 File: USPT Mar 19, 2002

DOCUMENT-IDENTIFIER: US 6358752 B1
TITLE: Liposome-enhanced test device and method

Abstract Text (1):
A test device and method for detecting or quantifying an analyte in a test sample employs an interdigitated electrode array and electroactive marker-encapsulating liposomes for signal generation and detection. The test device includes a contact portion on a first absorbent material, a capture portion either on the first absorbent material, or on a second absorbent material in fluid flow contact with the first absorbent material. The capture portion has a binding material specific for a portion of the analyte bound thereto. The device further includes an electrode array including first and second conductors each having a plurality of fingers, wherein the fingers of the conductors are interdigitated. The electrode array is positioned to induce redox cycling of an electroactive marker released either in or beyond the capture portion, depending upon whether direct (proportional) or indirect (inversely proportional) detection or measurement is desired. In the method of the invention, the test sample is applied to the contact portion, and allowed to migrate along the absorbent material(s) into the capture portion. Either before or after the migration, the test sample is contacted with a conjugate of liposomes and a second binding material for the analyte. To the extent that analyte is present in the sample, the conjugate is bound in the capture portion. By applying a voltage across the conductors, redox cycling of the marker is induced and a current is generated.

Brief Summary Text (2):
The present invention relates to a method for detecting or quantifying an analyte, and a test device used in the method. More particularly, the invention relates to a biosensor test device and method employing marker-loaded liposomes and electrochemical detection for signal amplification and quantitation.

Brief Summary Text (22):
The test device is employed in the method of the invention. In the method, the test sample is applied to the contact portion. Either before or after the application of the test sample, it is contacted and with a conjugate of electroactive marker-encapsulating liposomes and a second binding material. The second binding material is selected to bind with a portion of the analyte. The first binding material is selected to bind with a portion of the analyte other than the portion for which the second binding material is selected. The test sample and conjugate are incubated for a time sufficient to permit reaction between any analyte present in the sample and the second binding material.

Brief Summary Text (23):
The test sample is allowed to migrate from the contact portion toward and then into the capture portion. A voltage sufficient to induce redox cycling of the electroactive marker contained in the liposomes is applied across the conductors. After the test sample and the conjugate are incubated, liposomes bound in the capture portion are lysed to release the marker, which undergoes redox cycling as the result of the voltage applied across the conductors, causing current to flow between said first and second conductors. The presence or the amount of the resulting current is detected and correlated with the presence or amount, respectively, of the analyte in the test sample. In this embodiment, the magnitude of the current released from liposomes bound in the capture portion is directly proportional to the amount of analyte in the test sample.

Brief Summary Text (24):
In another embodiment of the invention, the electrode array is positioned to induce redox cycling of electroactive marker released from liposomes which migrate out of the capture portion. Thus, the presence or amount of the current generated by liposomes which are not bound in the capture portion is detected or measured. In this embodiment, the magnitude of the current generated is inversely proportional to the amount of analyte in the sample.

Brief Summary Text (26):
In addition, electroactive marker-loaded liposomes as used in the device and method of the invention provide a highly sensitive, rapid or even instantaneous signal production/amplification system. Furthermore, the amount of marker measured in the electrochemical measurement portion of the absorbent material of the test device is directly proportional to the analyte concentration in the sample. This feature of the invention provides a particular advantage over prior test devices, nucleic acid detection assays, and immunoassays, providing an intuitive correlation between signal strength and analyte concentration. Electrochemical detection offers greater sensitivity than colorimetric determination and is comparable to fluorimetry or chemiluminescence. In addition, the present invention provides quantitative results that can be obtained directly from the electroanalyzer or other detection instrumentation to which the test device is connected, without the need to transfer the device to a separate optical measurement device. Also, electrochemical detection allows for testing in solutions or mixtures that are highly colored or include particulate matter, and which, therefore, would interfere with optical detection.

Drawing Description Text (9):
FIG. 6 is a schematic depiction of a derivatized, marker-loaded liposome captured via the target sequence and capture probe in the capture portion of the test device of the present invention.

Drawing Description Text (12):
FIG. 9 is a graph of liposome-borne reporter probe/antisense reporter probe binding intensity versus reporter probe concentration.

Detailed Description Text (2):
The method of the invention employs two binding materials for the target analyte--one conjugated to marker-encapsulating liposomes, the other immobilized on a portion of an absorbent material. The two binding materials bind to different portions of the analyte. An excess of both the liposome conjugate and the immobilized binding material are employed. Thus, to the extent that the analyte is present in the test sample, the marker-loaded liposomes become bound to the absorbent material via the analyte. Thus, the test device and method of the invention rely on the "sandwich" formed by the first binding material (immobilized on the absorbent material), the analyte, and the second binding material (conjugated to the marker-loaded liposomes).

Detailed Description Text (3):
The invention encompasses both direct and indirect detection/measurement methods. In the former, the presence or amount of the marker bound in the immobilization, or "capture" portion of the test device is detected. In this embodiment, the amount of marker bound in the capture portion is directly proportional to the amount of analyte in the test sample. The indirect detection embodiment involves detecting or measuring the marker released from liposome conjugate which migrates beyond the capture portion, which is indirectly proportional to the amount of analyte in the test sample.

Detailed Description Text (7):
As is discussed in greater detail below, the method of the invention employs a conjugate of marker-encapsulating liposomes and a second binding material. The second binding material may be conjugated to the liposome surface. The second binding material must be bound to the liposomes so as to present a portion of the second binding material that may be recognized by the analyte.

Detailed Description Text (8):

Suitable conjugation methods are discussed in U.S. Pat. Nos. 5,789,154; 5,756,362; and 5,753,519. For example, the liposome surface may be activated with thiol groups and coupled to a maleimide group on the second binding material. Or, conversely, maleimide-activated liposomes and thiol group-activated binding material may be employed.

Detailed Description Text (16):
As described herein, the test device includes one or more absorbent materials. Regardless of the number of absorbent pads or materials employed, it is important that at least that portion of the test strip comprising and between the conjugate application and capture portions be made of a non-liposome lysing material. The material on which the first binding material is immobilized must be capable of supporting the immobilization, and the material(s) must allow liquid migration (lateral flow).

Detailed Description Text (17):
Absorbent materials having high surface areas (such as nitrocellulose) are particularly preferred for some applications in that the first binding material and, if desired, the liposome conjugate, may be supported on such materials in high concentrations. It is to be understood, however, that the concentrations of binding material and liposome conjugate which are actually used are dependent in part on the binding affinity of the first and second binding materials. Accordingly, the scope of the invention is not limited to a particular concentration of binding material on the absorbent material.

Detailed Description Text (18):
Application of the binding material and, if desired, the liposome conjugate to the absorbent material may be accomplished by well-known techniques, for example, by spraying or spotting solutions of these components onto the absorbent material.

Detailed Description Text (19):
The first binding material and/or liposome conjugate can be bound to the absorbent material by covalent bonding. For example, the material to be bound can be applied directly to the absorbent material, and then bonded thereto via ultraviolet radiation. Alternatively, materials can be adsorbed onto the absorbent material, as long as the binding of the first binding material to the absorbent material is non-diffusive. This will involve contacting the absorbent material with a solution containing the material to be bound to the material and allowing the material to dry. In general, this procedure will be useful only where the absorbent material is relatively hydrophobic or has a high surface charge, and subsequent treatment with proteins, detergents, polysaccharides, or other materials capable of blocking nonspecific binding sites will be required.

Detailed Description Text (21):
Before or after application of the binding material and, optionally, the liposome conjugate, to the appropriate portion(s) on the absorbent material(s), the residual nonspecific binding capacity of the absorbent material(s) can be, and preferably is, saturated or blocked with one or more types of proteins or other compounds such as polyvinylpyrrolidone, polyvinylalcohol, other suitable polymeric blocking agents etc., which do not specifically bind the materials to be employed in the assay. Blocking is generally carried out after the binding material and liposome conjugate are applied to the strip, but it may be possible to block the strip before these components are applied depending on the method of application, the particular blocking agent and absorbent material employed. Thus, for example, the residual binding capacity of the substrate may be blocked so as to prevent nonspecific binding by the use of bovine serum albumin, as described in Towbin, et al., Proc. Nat'l. Acad. Sci., 76 (1979) 4350, which is hereby incorporated by reference. The techniques for preventing non-specific binding are generally known in the art, and such techniques are also generally applicable to preventing nonspecific binding in the assay of the present invention. Examples of particularly suitable techniques for blocking with polyvinylpyrrolidone and polyvinylalcohol are described, for example, Bartles, et al. Anal. Biochem., 140 (1984) 784, and in British Patent Specification GB 2204398 A, respectively, which are hereby incorporated by reference. Alternatively, one or more blocking agents can be incorporated into the buffer solution used to wash or carry test components along the absorbent material(s).

Detailed Description Text (22):
In conjunction with a blocking agent or agents, a surfactant may be applied to the absorbent material in a concentration sufficient to promote homogeneous flow of the test solution across the test device, to facilitate migration of the liposome conjugate without lysis of the liposomes. Suitable surfactants include Brij.TM. (polyoxyethylene ether), Tween 20.TM. (polyoxyethylenesorbitan monolaurate), Triton X-100.TM. (t-octylphenoxypolyethoxyethanol), sodium dodecylsulfate, n-octyl--D-glucopyranoside, Span 20.TM., Nonindet P-40, Chapso.TM., Turgitol.TM. and sodium dioxycholate. The concentration of the surfactant(s) employed in a blocking solution will depend, in part, upon the liposome composition. In general, surfactants may be incorporated in a concentration of from about 0 to about 0.01 volume percent of the blocking solution, preferably from about 0.001 to about 0.005 volume percent of the blocking solution. It is important that the concentration of surfactant applied to the absorbent material be controlled, as premature lysis of the liposomes may occur if the surfactant concentration is too high. Tween 20.TM. is a preferred surfactant for use in a blocking solution.

Detailed Description Text (24):
The test device preferably comprises two or three absorbent pads, laid end-to-end, as discussed more fully below. In the two pad embodiment, the first pad includes both the contact portion and the capture portion, which is preferably begins at or beyond about half-way along the strip, to allow sufficient space on the pad in front of the capture zone for reaction or hybridization of the target with the second binding material carried on the liposomes. A second pad may be employed as a wicking pad, as discussed more fully below, to pull excess reagents out of the first absorbent pad. If three pads are employed, the capture portion is preferably located on the center pad, most preferably at or near the center of the pad. In this embodiment, the wicking pad is the third pad, but an additional pad or pads could be used as wicking pads beyond a third pad.

Detailed Description Text (28):
Alternatively, the liposome-second binding material conjugate can be introduced onto the absorbent material in the contact portion, at the same location as the test sample or at a separate location. A third alternative involves introducing the liposome conjugate just before or directly onto the capture portion of the test device.

Detailed Description Text (29):
The migration of the test sample and liposome conjugate, if introduced outside the capture portion, is preferably assisted by introducing a wicking reagent, preferably a buffer solution, onto the strip to carry the test components along the strip. Alternatively, if the sample volume is sufficiently large, it is not necessary to employ a separate buffer solution. If the liposome conjugate is applied directly to the capture portion, the buffer solution is preferably introduced onto the strip following a period of incubation to allow reaction or hybridization of any analyte present in the test sample and the conjugate.

Detailed Description Text (30):
In constructing the test devices in accordance with the invention, it is desirable to position the capture portion relatively close to the contact portion in order to minimize the time necessary for the test mixture to reach and pass through the capture portion. However, if the test sample has not been incubated with the liposome conjugate prior to introduction onto the test device, it is important that the capture portion and the contact portion be separated sufficiently so as to provide sufficient opportunity for any analyte present in the test sample and the second binding material conjugated to the liposomes to bind to one another so that the conjugate which correlates to the amount of analyte in the test sample ultimately becomes bound in the capture portion via the analyte and the first binding material.

Detailed Description Text (31):
FIG. 1 shows a 3-pad test device in accordance with the direct measurement embodiment of the invention. It includes first, second, and third absorbent materials 104, 106, and 108, respectively, resting on support 102. Each of these absorbent materials, which are also identified functionally herein as the sample pad, reaction pad, and

wicking pad, respectively, is in fluid flow contact with the adjacent absorbent pad. Sample pad 104 includes the contact portion, where the test sample and the liposome conjugate containing solution or mixture are applied. The reaction pad includes capture portion 110, to which the first binding material is non-diffusively bound.

Detailed Description Text (32):
As the test sample and conjugate mixture migrate across the device from sample pad 104 into reaction pad 106, any analyte present in the test sample binds with the second binding material conjugated to the liposomes. Because the second binding material is selected to bind with only a portion of the analyte, the analyte also remains available for binding with the first binding material, as the test components migrate into capture portion 110. In this way, a quantity of marker-loaded liposomes which is proportional to the concentration of the analyte in the test sample becomes bound in the capture portion of the test device.

Detailed Description Text (33):
In FIG. 1, interdigitated electrode array 112, shown in greater detail in FIGS. 3a and 3b, is shown removed from its position under capture portion 110. When in place under capture portion 110, electrode array 112 is positioned to induce redox cycling of the electroactive marker released from the liposomes bound via the analyte in capture portion 110 upon lysis of the liposomes and passage of the test solution containing the released marker through absorbent material 106.

Detailed Description Text (34):
Binding among the liposome conjugate, analyte and first binding material is depicted in FIG. 6, which is intended to depict, in particular the preferred embodiment where the analyte is a target nucleic acid sequence. In this embodiment, the first binding material is capture probe 514, which is selected to, and does, hybridize with a portion of target nucleic acid sequence 516. Capture probe 514 is immobilized in the capture portion of absorbent material 510. Second binding material 520, referred to herein as a reporter probe for the nucleic acid detection/measurement embodiment, is selected to, and does, hybridize with a portion of target nucleic acid sequence 516 other than that portion of the target with which capture probe 514 hybridizes. Second binding material 520 is conjugated to marker-encapsulating liposome 518, and binds the marker-encapsulating liposome via target nucleic acid sequence 516 and capture probe 514 to absorbent material 510 in the capture portion. Interdigitated electrode array 512 is positioned to induce redox cycling of the marker released from liposome 518 upon lysis.

Detailed Description Text (35):
A separate absorbent pad may be employed as a wicking pad, regardless of how many other absorbent pads are employed. The wicking pad serves both to pull the liquid sample and the liposome conjugate along the test strip formed by the absorbent pads, and to pull unbound conjugate out of the capture portion to enhance assay accuracy. The wicking material and pad length are preferably matched to the other components of the device and the particular test components employed in order to provide sufficient fluid flow contact along the test strip. A preferred wicking material is Whatman filter paper.

Detailed Description Text (38):
FIGS. 2a and 2b show assembled and exploded views of a cassette-enclosed test device in accordance with the invention. The cassette functions to hold the test strip in proper position and to position and support the interdigitated electrode array, as well as any protective barriers or screens protecting the electrode fingers from damage which might otherwise result from direct contact with the absorbent material of the test device. The device includes top housing 202 and bottom housing 222, which may conveniently be fabricated from plastic, for example, via injection molding techniques. Test sample and liposome conjugate may be introduced onto absorbent material 232 via buffer solution port 204 and test mixture port 206, respectively. Progress of the migration of the test components can be confirmed via opening 216.

Detailed Description Text (39):
Test accuracy can be enhanced by isolating the capture portion from the remainder of the test strip once the reaction mixture has migrated into the capture portion and the unbound conjugate has flowed past the capture portion, into a separate wicking

pad, for example. For this purpose, the device shown in FIGS. 2a and 2b further includes cutting device 212 and cutting device receiver 218. Upon completion of the reaction between the analyte-liposome conjugate and the second binding material in the capture portion of the absorbent material, cutting device 212 can be used to cut through the absorbent material around the perimeter of the capture portion to physically isolate the capture portion from the adjacent portion of the absorbent material in order to ensure that only marker released from the liposomes bound via the analyte in the capture portion is measured via interdigitated electrode array 224. As cutting device 212 is pushed into cutting device receiver 218, the capture portion of the absorbent material is dropped onto gasket 220, which forms a seal preventing leakage of the liquid carried with the capture portion.

Detailed Description Text (41):
A voltage is applied across leads 226 and 228 to electrode array 224, which induces redox cycling of the electroactive marker released as the result of lysis of the liposomes bound to the capture portion. Liposome lysis may be achieved by introducing a liposome lysing agent through the opening in cutting device 212, preferably after the capture portion has been isolated from the adjacent absorbent material. As an alternative to application of the liposome solution or suspension at the time the assay is run, the liposome conjugate may be incorporated directly into the test strip, for example, in a dehydrated state, or dried onto the surface of the interdigitated electrode array. In these embodiments, the test mixture or test buffer provides the liquid to solubilize the lysing agent, facilitating its contact with the liposomes. The used of a liposome lysing agent in dry form avoids the addition of an additional test reagent during the assay.

Detailed Description Text (52):
The position of the contact and capture portions should be governed by the basic principle involved in the present invention. For example, whether the test sample and conjugate are applied to the same or separate locations in the contact portion of the test device, one desires to provide sufficient opportunity for binding to occur between the second binding material conjugated to the liposomes and any analyte present in the test sample so that the concentration of the conjugate bound in the capture portion accurately reflects the concentration of the analyte in the test sample. Generally speaking, if nitrocellulose having a pore size of 8 .mu.m is employed for the first or first and second absorbent materials, the distance between the contact portion and the capture portion should range from about 5 mm to about 20 .mu.m. If several capture portions are used for multi-analyte determinations, the capture portions can be grouped close together or apart but must not be so close as to compromise resolution of the signals. Consequently, such measurement portions usually should be spaced not less than 0.5 mm apart, preferably at least 1 mm apart.

Detailed Description Text (53):
The test sample may be derived from a wide variety of sources, such as physiologic fluids, illustrated by saliva, sweat, serum, plasma, urine, tear fluid, spinal fluid, etc., chemical processing streams, food, waste water, natural waters, soil extracts, etc. In carrying out the method of the invention, the sample suspected of containing the analyte may be combined with the conjugate in an electrolytic aqueous medium to form an aqueous test mixture or solution. Various addenda may be added to adjust the properties of the test mixture, or of a carrier solution used as a wicking reagent, depending upon the properties of the other components of the device, as well as on those of the liposomes or the analyte analog-liposome conjugate, or the analyte itself. Examples of solution addenda which may be incorporated into test, control, or carrier solutions or mixtures in accordance with the invention include buffers, for example, pH and ionic strength, sample or analyte solubilizing agents, such as, for example, nonpolar solvents, and high molecular weight polymers such as Ficoll.RTM., a nonionic synthetic polymer of sucrose, available from Pharmacia, and dextran.

Detailed Description Text (57):
The conjugate of the second binding material and the marker-encapsulating liposomes may be prepared by procedures generally known in the art, with the particular procedure used in a given case being dependent upon the liposome components and binding material employed. Such techniques include covalent coupling, derivatization or activation, and the like. The liposomes may be produced from a component which has been derivatized with the second binding material, whereby the liposomes, when

produced, are conjugated with the second binding material. In another procedure, the liposomes, including the marker, may be initially formed, followed by conjugating the liposomes with the second binding material by procedures known in the art.

Detailed Description Text (58):
Liposomes can be prepared from a wide variety of lipids, including phospholipids, glycolipids, steroids, relatively long chain alkyl esters; e.g., alkyl phosphates, fatty acid esters; e.g. lecithin, fatty amines, and the like. A mixture of fatty materials may be employed, such as a combination of neutral steroid, a charge amphiphile and a phospholipid. Illustrative examples of phospholipids include lecithin, sphingomyelin, and dipalmitoylphosphatidylcholine, etc. Representative steroids include cholesterol, chlorestanol, lanosterol, and the like. Representative charge amphiphilic compounds generally contain from 12 to 30 carbon atoms. Mono- or dialkyl phosphate esters, or alkylamines; e.g. dicetyl phosphate, stearyl amine, hexadecyl amine, dilaurylphosphate, and the like are representative.

Detailed Description Text (59):
The liposome sacs are prepared in aqueous solution containing the marker whereby the sacs will include the electroactive marker in their interiors. The liposome sacs may be prepared by vigorous agitation in the solution, followed by removal of the unencapsulated marker. Further details with respect to the preparation of liposomes are set forth in U.S. Pat. No. 4,342,826 and PCT International Publication No. WO 80/01515, both of which are incorporated by reference.

Detailed Description Text (60):
As hereinabove indicated, the signal producing system includes an electroactive marker included in the interior of the conjugated liposomes. Suitable markers are those which are electrochemically active but will not degrade the liposomes or otherwise leach out of the liposomes. They include metal ions, organic compounds such as quinones, phenols, and NADH, and organometallic compounds such as derivatized ferrocenes. A reversible ferrocyanide-ferricyanide couple is the most preferred electroactive marker in accordance with the invention. An equal mixture of ferrocyanide and ferricyanide is particularly preferred.

Detailed Description Text (61):
The use of liposomes as described in the present application provides several advantages over traditional signal production systems employing, for example, enzymes. These advantages include increased signal intensity, shelf stability, and instantaneous release of signal-producing markers, as described in T. A. Siebert, S. G. Reeves, R. A. Durst, Analytica Chimica Acta 282, 297-305 (1993); W. T. Yap, L. Locascio-Brown, A. L. Plant, S. J. Choquette, Analytical Chemistry 63, 2007 (1991); A. L. Plant, M. V. Brizgys, L. Locasio-Brown, R. A. Durst, Analytical Biochemistry 176, 420-426 (1989); L. Locascio-Brown, A. L. Plant, V. Horvath, R. A. Durst, Analytical Chemistry 62, 2587-2593 (1990); and R. A. Durst, L. Locascio-Brown, A. L. Plant, R. D. Schmid, Eds., Flow Injection Analysis based on enzymes or antibodies, vol. 14 (VCH, Weinheim, 1990), each of which is hereby incorporated by reference. For example, initial calculations indicate that the rupture of a single liposome in a typical capillary electrophoresis sample volume would lead to a concentration of 5 .mu.M K.sub.4 Fe(CN).sub.6 at the interdigitated electrode array detector. Therefore, due to the great sensitivity of the interdigitated electrode arrays, the detection of single liposome events should be theoretically possible with the present system.

Detailed Description Text (62):
As described above, lysis of the liposomes in the capture portion may be accomplished by applying a liposome lysing agent to the capture portion of the absorbent material after the conjugate becomes bound therein. Suitable liposome lysing materials include surfactants such as octylglucopyranoside, sodium dioxycholate, sodium dodecylsulfate, polyoxyethylenesorbitan monolaurate sold by Sigma under the trademark Tween-20, and a non-ionic surfactant sold by Sigma under the trademark Triton X-100, which is t-octylphenoxypolyethoxyethanol. Octylglucopyranoside is a preferred lysing agent for many assays, because it lyses liposomes rapidly and does not appear to interfere with signal measurement. Alternatively, complement lysis of liposomes may be employed, or the liposomes can be ruptured with electrical, optical, thermal, or other physical means.

Detailed Description Text (63):
The movement of the test components along the absorbent material(s) is due to capillary action. This capillary movement along the absorbent material causes the test mixture to be carried to and through the capture portion, where measurement of the marker released from the liposomes takes place.

Detailed Description Text (64):
An electroactive species, such as ferrocyanide, is encapsulated in the liposomes. The interdigitated electrode array is positioned to induce redox cycling of an electroactive marker released in the capture portion.

Detailed Description Text (74):
In the method of the invention, a fixed voltage is applied across the conductors to induce redox cycling of the electroactive marker released from the liposomes captured in the capture portion. A simple battery can be used to apply the voltage. Other devices which may be used as potentiostats in accordance with the invention include the Cypress (Lawrence, Kans.) System Electrochemical Analyzer (CS-1090) and the BAS (West Lafayette, Ind.) Amperometric Detector (LC-4C, LC-3C, LC-3D).

Detailed Description Text (77):
The concentration of electrolytes in the medium will usually be adjusted to achieve isotonicity or equi-osmolality with the solution in the interior of the liposomes to prevent their crenation or swelling.

Detailed Description Text (78):
With some increased complexity of the excitation waveform applied by the electroanalyzer, electrochemical measurement in accordance with the invention may also be carried out using stripping voltammetry, employing, for example, liposome encapsulated metal ions for detection and measurement.

Detailed Description Text (82):
As a matter of convenience, the present device can be provided in a kit in packaged combination with predetermined amounts of reagents for use in assaying for an analyte or a plurality of analytes. Aside from the absorbent test device and the liposome conjugate, other additives such as ancillary reagents may be included, for example, stabilizers, buffers, and the like. The relative amounts of the various reagents may be varied widely, to provide for concentration in solution of the reagents which substantially optimize the sensitivity of the assay. The reagents can be provided as dry powders, usually lyophilized, including excipients, which on dissolution will provide for a reagent solution having the appropriate concentrations for performing the assay. The kit or package may include other components such as standards of the analyte or analytes (analyte samples having known concentrations of the analyte).

Detailed Description Text (117):
Liposome Preparation

Detailed Description Text (118):
Liposomes were prepared following the procedures described in U.S. Pat. Nos. 5,789,154; 5,756,362; and 5,753,519; and U.S. patent application Ser. No. 08/722,901, the disclosures of which are hereby incorporated by reference. Oligonucleotides, specific for the target sequence, were covalently coupled to the outside of the liposome membrane.

Detailed Description Text (126):
Subsequently, as soon as all sample buffer migrated along the strip, the capture portions was used for detection. In one trial, the capture portion was cut out and laid on top of an IDUA, a detergent was added and the signal detected after a few minutes. In an alternative trial, the capture portion was cut out and laid on top of an IDUA which had dried detergent on its surface, and the signal was detected after a few minutes. When sulforhodamine B (SRB) encapsulating liposomes were used, the signal was detected using a scanner.

Detailed Description Text (127):
Different concentrations of a synthetic target sequence were detected using a 2-pad assembly, with introduction of liposomes directly onto the capture portion. The

results are shown in FIG. 11.

Detailed Description Text (130):
FIG. 9 shows the results of an investigation of binding intensity for a reporter probe-antisense reporter probe pair at various concentrations of reporter probe on the liposome surface. The antisense reporter probe was immobilized on the capture portion of an absorbent pad. Optimization of the concentration of the second binding material on the liposomes requires consideration of a variety of factors. For example, a sufficient concentration of binding material must be employed to achieve good binding intensity, without sacrificing assay sensitivity. In addition, the concentration of the second binding material on the liposomes must be sufficient to accommodate the concentration of the analyte in the test sample, for example, to avoid the "hook effect" which could otherwise result from a high concentration of analyte.

Other Reference Publication (1):
Rule et al., "Rapid Method for Visual Identification or Specific DNA Sequences Based on DNA-Tagged Liposomes", Clin. Chem., 42(8):1206-1209 (1996).

Other Reference Publication (2):
Rule et al., "Characteristics of DNA-Tagged Liposomes Allowing Their Use in Capillary-Migration, Sandwich-Hybridization Assays", Anal. Biochem., 244:260269 (1997).

Other Reference Publication (5):
Durst et al., "Development of Liposome-Enhanced Immuno-Biosensing Devices for Field Measurements of Toxic Sybstances", 2.sup.nd Bioelectroanalytical Symposium, 15-32 (1992).

CLAIMS:

1. A method for detecting or quantifying an analyte in a liquid test sample, comprising:

providing a test device comprising:

a contact portion on a first absorbent material;

a capture portion either on said first absorbent material, or on a second absorbent material in fluid flow contact with said first absorbent material, wherein said capture portion has a first binding material bound to said capture portion; and

an electrode array comprising a first conductor having a plurality of fingers, and a second conductor having a plurality of fingers, wherein said fingers of said first conductor are interdigitated with said fingers of said second conductor, said first and second conductors are electrically connected to one another via a voltage source and readout device, and said array is positioned to induce redox cycling of an electroactive marker released in said capture portion;

applying the test sample to said contact portion;

applying a voltage across said conductors, wherein said potential is sufficient to induce redox cycling of said marker;

allowing the test sample to migrate from said contact portion into said capture portion;

contacting the test sample with a liposome conjugate of liposomes and a second binding material, wherein said liposomes encapsulate an electroactive marker, wherein said second binding material binds with a portion of the analyte; and wherein said first binding material binds with a portion of the analyte other than the portion of the analyte for which the second binding material is selected;

incubating the test sample with the conjugate for a time sufficient to permit reaction between any analyte present in the test sample and the second binding

material;

after said incubating and said allowing, lysing any liposomes present in said capture portion to release said marker, whereby said marker undergoes redox cycling induced by said conductors causing current to flow between said first and second conductors;

detecting the presence or amount of said current; and

correlating the presence or amount of said current with the presence or amount, respectively, of the analyte in the test sample.

2. A method for detecting or quantifying an analyte in a liquid test sample, comprising:

providing a test device comprising:

a contact portion on a first absorbent material;

a capture portion either on said first absorbent material, or on a second absorbent material in fluid flow contact with said first absorbent material, wherein said capture portion has a first binding material bound to said capture portion; and

an electrode array comprising a first conductor having a plurality of fingers, and a second conductor having a plurality of fingers, wherein said fingers of said first conductor are interdigitated with said fingers of said second conductor, said first and second conductors are electrically connected to one another via a voltage source and readout device, and said array is positioned to induce redox cycling of an electroactive marker released from liposomes which migrate beyond said capture portion;

applying the test sample to said contact portion;

applying a voltage across said conductors, wherein said potential is sufficient to induce redox cycling of said marker;

allowing the test sample to migrate from said contact portion through said capture portion;

contacting the test sample with a liposome conjugate of liposomes and a second binding material, wherein said liposomes encapsulate an electroactive marker, wherein said second binding material binds with a portion of the analyte; and wherein said first binding material binds with a portion of the analyte other than the portion of said analyte for which said second binding material is selected;

incubating the test sample with the conjugate for a time sufficient to permit reaction between any analyte present in the test sample and the second binding material;

after said incubating and said allowing, lysing any liposomes which migrate beyond said capture portion to release said marker, whereby said marker undergoes redox cycling induced by said conductors causing current to flow between said first and second conductors;

detecting the presence or amount of said current; and

correlating the presence or amount of said current with the presence or amount, respectively, of the analyte in the test sample, wherein the presence or amount of said current is inversely proportional to the presence or amount, respectively, of the analyte in the test sample.

5. A method according to claim 1, wherein said lysing is carried out by introducing a liposome lysing agent onto said capture portion after said allowing and said incubating.

10. A method according to claim 1, wherein said contacting is carried out by applying

the test sample and the liposome conjugate to said absorbent material and allowing the test sample or the conjugate to migrate into contact with the other.

11. A method according to claim 1, wherein said contacting is carried out by reversibly immobilizing the liposome conjugate on said absorbent material between said contact portion and said capture portion, and allowing the test sample to migrate through the immobilized conjugate toward said capture portion.

13. A method according to claim 1, wherein the liposomes are prepared from one or more phospholipids, glycolipids, steroids, alkyl phosphates, or fatty acid esters.

21. A test device for detecting or quantifying an analyte in a liquid test sample, said test device comprising:

a contact portion on a first absorbent material;

a capture portion either on said first absorbent material, or on a second absorbent material in fluid flow contact with said first absorbent material, wherein said capture portion has a binding material specific for the analyte bound to said capture portion; and

an electrode array comprising a first conductor having a plurality of fingers, and a second conductor having a plurality of fingers, wherein said fingers of said first conductor are interdigitated with said fingers of said second conductor, and wherein said electrode array is positioned to induce redox cycling of an electroactive marker released from liposomes which migrate out of said capture portion.

L2: Entry 12 of 51 File: USPT Sep 11, 2001

DOCUMENT-IDENTIFIER: US 6288027 B1
TITLE: Preserving a hemoglobin blood substitute with a transparent overwrap

Brief Summary Text (5):
Previously, at least four other types of blood-substitutes have been utilized, including perfluorochemicals, synthesized hemoglobin analogues, liposome-necapsulated hemoglobin, and chemically-modified hemoglobin. However, many of these blood-substitutes have typically had short intravascular retention times, being removed by the circulatory system as foreign substances or lodging in the liver, spleen, and other tissues. Also, many of these blood-substitutes have been biologically incompatible with living systems.

Brief Summary Text (37):
Acceptable isotonic solutions are as known in the art and include solutions, such as a citrate/saline solution, having a pH and osmolarity which does not rupture the cell membranes of RBCs and which displaces the plasma portion of the whole blood. A preferred isotonic solution has a neutral pH and an osmolarity between about 285-315 mOsm. In a preferred embodiment, the isotonic solution is composed of an aqueous solution of sodium citrate dihydrate (6.0 g/l) and of sodium chloride (8.0 g/l).

WEST

Generate Collection Print

L2: Entry 16 of 51 File: USPT Mar 27, 2001

DOCUMENT-IDENTIFIER: US 6207456 B1
TITLE: Nucleic acid delivery vehicle

Abstract Text (1):
A composition includes a liposome which has a polynucleic acid and a peptide capable of disrupting membranes under acidic conditions encapsulated within it. The composition is used for efficient transfer of nucleic acids into cells both in vitro as well as in vivo.

Brief Summary Text (5):
Another method for in vivo gene transfer uses a complex of positively charged liposomes (composed of synthetic lipids) that bind to the DNA (the DNA is not encapsulated in the liposomes) (Zhu, N., et al., Science, 261:209-211, 1993). However, the toxicity of positively charged liposomes (Raz, E., et al., In: Vaccines, Cold Spring Harbor Laboratory Press. Cold Spring Harbor, N.Y., 1994) limits the use of this method.

Brief Summary Text (6):
A complex of poly-L-lysine coupled to DNA and a ligand which can be targeted to the cell surface was also used for in vivo gene transfer (Wu, G. Y. and Wu, C. H., J. Biol. Chem., 263:14621-14624, 1988; Perales, J. C., et al., Proc. Natl. Acad. Sci., USA 91:4086-4090, 1994). However, transient expression of the transferred gene and immunogenicity of the complex limits the use of this method. Neutral or negatively charged liposomes (composed of synthetic or natural lipids) that encapsulate DNA were hitherto used for in vivo gene transfer (Nicolau, C., et al., Proc. Natl. Acad. Sci., USA, 80:1060-1072, 1983; Liebiger, B., et al., Biochem. Biophys. Res. Commun., 174:1223-1231, 1991).

Brief Summary Text (7):
Such liposomes are non-toxic, non-immunogenic and biode-gradable (Storm et al. In: Gregoriadis, G. (Ed), Liposome Technology, 2nd Ed. CRC Press, Baca Rator, Fla. 1993, 00.345-383) and are therefore good candidates for repetitive high dose treatment. However, the gene transfer efficiency in these experiments was low. One possible explanation for the low efficiency is that the DNA, which is encapsulated in liposomes and enters the cytoplasm through the lysosome (Cudd, A., and Nicolau, C., Biochim. Biophys. Acta, 845:477-491, 1985), is degraded by active DNases in the lysosome.

Brief Summary Text (8):
Therefore, treatments that inhibit lysosome activities can increase the amount of liposome-encapsulated DNA inside the cells.

Brief Summary Text (10):
The efficiency of cationic-liposome mediated in vitro DNA transfection into cells was also shown to be increased when the cationic-liposomes were mixed with the DNA to be transfected together with two additional peptides derived from the Influenza virus hemagglutinin protein to form a complex between these three components (Kamata, H. et al. Nuclear Acid Research 22:536-537, 1994).

Brief Summary Text (11):
Fusion of liposomes prepared by reverse-phase evaporation (RPE) carrying DNA or protein molecules to target cells was shown to be mediated by Sendai virus proteins

(Kameda, Y. et al, Exp. Cell Res. 173:56, 1987) or influenza virus proteins (Lapidot, M., Loyter, A., Exp. Cell Res. 189:241-246, 1990; Tikchonenko, T. I. et al, Gene 63:321-330, 1988) which were introduced into the liposome membrane (i,e, were present on the liposome's outer surface). The introduction of the DNA or protein encapsulated in the liposome was dependent on the presence of an active viral fusion protein and, therefore, either intact Sendai or influenza virus particles or their reconstituted envelopes were required. Liposomes containing modified reconstituted viral envelopes integrated in their lipid bilayer (Gould-Fogerite, S. et al, Gene, 84, 429-438, 1989) were also prepared by the protein-cochleate technique. Such liposomes were used for stable gene transfer and expression in animals.

Brief Summary Text (12):
All the above mentioned liposomes have large viral particles or reconstituted viral envelopes integrated in their lipid membrane which protrude outwards from the liposome membrane. In vivo administration of liposomes carrying such large viral particles may be hindered by the high antigenicity of such large particles as well as occasionally by their toxic effects.

Brief Summary Text (16):
The invention concerns a novel composition for liposome-mediated ex vivo (particularly to cells removed from the body and returned to the body following a genetic manipulation) and in vivo transfer of nucleic acid sequences to cells. The formulation comprises liposomes in which a nucleic acid sequence is co-capsulated with a short peptide that disrupts membranes at a low pH.

Brief Summary Text (19):
ii. a liposome; and

Brief Summary Text (21):
said peptide and the polynucleic acid molecule being encapsulated within the liposome.

Brief Summary Text (22):
The polynucleic acid molecule may be a DNA molecule, an RNA molecule, a moleclue consisting of both ribonucleotides and deoxy-nucleotides (RNA/DNA hybrid). The polynucleic acid molecule may be a large nucleic acid construct, such as a plasmid, an oligonucleotide, etc. The polynucleic acid is typically encapsulated within the aqueous interior of the liposome.

Brief Summary Text (23):
The liposomes used in accordance with the invention may be multilamellar or unilamellars. Most types of liposomes belong to either one of the following three types: multilamellar vesicles (MLV), small unilamellar vesicles (SUV) and large unilamellar vesicles (LUV). MLVs typically form spontaneously upon hydration of dried phospholipids. SUVs, may be formed from MLVs by sonication and unlike the multilayered, onion skin-like structure of MLVs, they are single layered. SUVs are small with a high surface-to-volume ratio and thus have the lowest capture volume of aqueous space per weight of lipid.

Brief Summary Text (25):
The liposomes may be comprised of a variety of lipids, including phospholipids, glycolipids, etc. Preferably phospholipids constitute a major component in the liposomes' membranes. Preferred phospholipids are .alpha.-lecithines (also known as phosphatidyl-cholines), which are mixtures of diglyceride of stearic, palmitic and oleic acids linked to the choline ester of phosphoric acid. Lecithines are found in and obtainable from animals and plants. Preferred sources of lecithines are eggs, soybeans, animal tissues such as brain, heart, and the like. Lecithines can also be produced synthetically. As will no doubt be appreciated by the artisan, the source of the phospholipid is immaterial to the present invention and any phospholipid will likely be suitable.

Brief Summary Text (27):
In addition to phospholipids, the liposomes may also comprise various other lipophilic or amphophilic molecules. The composition of the lipid membrane may be tailored for a variety of specific uses, either to obtain certain stability, size

distribution, etc.

Brief Summary Text (29):
The compositions of the inventions are preferably dehydrated, rehydrated vesicles (DRV). DRVs are prepared by rehydration of a dehydrated composition which upon addition of water spontaneously forms the composition of the invention. DRVs may be prepared in a number of ways. By one exemplary way, a mixture is first prepared consisting of SUVs, said agent, the polynucleic acid molecule and cryoprotectants. Examples of cryoprotectants are sucrose and amino acid (e.g. hystidine, lysine and arginine). The cryoprotectants are typically added at an osmolarity which is less than physiological (i.e. less than about 300 mOsm). The mixture is then lyophilized up to a water content of less than 2%. The reconstitution is typically a multi-stage procedure wherein the first step is a low volume rehydration, i.e., rehydration with a volume of an aqueous solution (which may be water or preferably an aqueous solution comprising salts (e.g. NaCl) or other solutes (e.g. sugar) to yield isotonicity with body fluids, i.e. osmolarity of about 300 mOsm), equal to about a third or less than the final water volume. Typically, as noted above, the compositions of the invention comprises, on a weight per volume basis, about 10% lipids and a low volume of the aqueous solution in the first hydration step, is typically addition of water to yield a concentration of lipids of about 30% (w/v). Then aqueous solution is further added gradually to yield the final volume.

Brief Summary Text (31):
The manner of preparation of liposomes and their tailoring to suit a specific need is generally known to the artisan and is outside the scope of the present writing. Non-limiting examples of methods for the preparation of liposomes are those mentioned above being reverse-phase evaporation (RPE) (Kameda et al, supra) or the protein-cochleate technique (Gould-Fagerite et al, supra).

Brief Summary Text (32):
The liposome of the invention may be targeted to a desired cell by various targeting methods known per se, such as by having a recognition molecule anchored in the liposome membrane, e.g. a member of a binding couple, the other member being one molecule on the surface of the target cell (examples of such binding couples are antibody-antigen, the antibody being anchored within the cell, and the antigen being antigen specifying the target cell; ligand-receptor couple, ligand being anchored in the liposome membrane and the receptor being a specific receptor for the target cell; sugar-lectin couple, the lectin being anchored in the cell membrane and the sugar being displayed on a cell surface; etc.).

Brief Summary Text (33):
The peptide comprised in the composition of the invention is a small molecule comprising up to about 50 amino acids (a.a.). In contrast to prior art liposomes which incorporate large viral particles in their membrane having at least a portion protruding outwards from the liposome surface, the peptide of the present invention is encapsulated within the liposome wherein it is either incorporated within the lipid layer of the liposome or is encapsulated in the liposomes inner aqueous part. Therefore, in vivo administration of a composition comprising a liposome of the invention does not elicit an immunogenic reaction and the composition also has no toxic effects.

Brief Summary Text (34):
The peptide of the invention is such which changes its conformation under acidic conditions to conformation where it becomes active in disrupting membranes. Such a peptide typically undergoes a conformational change under acidic conditions to a conformation of an .alpha.-helix where it becomes membrane disruptive. A liposome is internalized into a cell by endocytosis, and eventually reaches the lysosome within the cell. Owing to the acidic conditions within the lysosome, the peptide becomes membrane disruptive and following disruption of the lysosome's membrane the DNA is released into the cytoplasm.

Brief Summary Text (37):
(a) encapsulating a nucleic acid molecule within a liposome together with a peptide capable of disrupting membranes under acidic conditions; said peptide being comprised of less than about 50 amino acids; and

Brief Summary Text (38):
(b) delivering said liposome to the cells.

Drawing Description Text (2):
FIG. 1 shows results of an experiment demonstrating the presence of plasmid DNA in mouse liver 7 days post-injection of liposome encapsulated plasmid DNA. Balb/c mice (8 weeks old) were injected i.v. through the tail vein with 500 .mu.g of factor IX expression vector (pCI-FIX) encapsulated in liposome with (lane 4) or without (lane 3) a 22 mer peptide. Total DNA (30 .mu.g) purified from livers that were excised 7 days after the injection or from untreated mouse (lane 5), were digested with BamHI loaded on a 1% agarose gel and subjected to Southern blot analysis probed with .sup.32 P random priming-labelled Bluescript plasmid DNA. The number above each lane indicated the amount of plasmid DNA calculated according to 500, and 10 pg of the plasmid pCI-FIX (lane 1 and 2) loaded on the same gel.

Detailed Description Text (2):
1. Encapsulation of membrane disrupting peptide in liposomes

Detailed Description Text (6):
The free peptide and the peptide attached to palmitic acid were encapsulated in liposomes composed of egg phosphatidylcholine (EPC) by the freeze-drying method (Kirby and Gregoriadis in: Gregoriadis, G(Ed) Liposome Technology, CRC Press, Boca Raton, Fla., 1984, pp.19-24): EPC supplemented with 0.11% (molar ratio) DL-.alpha.-tocopherol (Lipoid) was dissolved to 20% (w/v) in tert-butanol and lyophilized. The dry lipid was resuspended to 10% (w/v)) in H.sub.2 O and homogenized, first in a high-shear mixer (Kinematica) and then in a high-pressure homogenizer (Rannie). The resulting solution contained small unilamellar vesicles (SUV), 30-50 nm in size as measured by particle analyzer (Counter Electronics). The free peptide or the peptide attached to palmitic acid was dissolved in 50 mM NaHCO.sub.3 and mixed with the SUV suspension at a ratio of either 1 or 2.5% molar ratio of peptide to lipid. The mixture was shell-frozen to a thin layer in siliconized glass vials in a dry ice/ethanol bath and lyophilized. Before use, the dry lipids and peptide were hydrated in two stages. First, H.sub.2 O was added to 30% of the final volume and the mixture was shaken until a homogeneous solution was obtained. The solution was then adjusted to 10% (w/v) lipid and 155 mM NaCl. Following hydration, a portion of the liposome solution was centrifuged at 15,000.times.g for 10 min. at 4.degree. C., and the supernatant containing free peptide was separated from the liposome pellet. The pelleted liposomes were then washed with 2 volumes of 155 mM NaCl and centrifuged as above. The peptide content in each fraction was determined by measuring adsorption of the peptide at 280 nm and then percentage of encapsulation was calculated. The results are summarized below in Table I.

Detailed Description Text (7):
These results indicate that encapsulation of the 22 mer peptide in liposomes is the highest at a 1% molar ratio of peptide to lipids, and that a higher molar ratio results in lower encapsulation level. In addition, free peptide was encapsulated more efficiently than peptide attached to palmitic acid.

Detailed Description Text (8):
2. Co-encapsulation of DNA and peptide in EPC liposomes

Detailed Description Text (9):
Three hundred and fifty micrograms of factor IX expression vector pCI-FIX (EcoRI-BamHI fragment of the plasmid pLIXSNL-2 (Gerrard et al., Nature Genetic 3:180-183 (1993) containing human factor IX cDNA inserted into the EcoRI and BamHI sites of the expression vector pCI (Promega, USA)) was mixed with 10 ng of nick translated factor IX expression vector labeled with .alpha.-.sup.32 P dATP. The DNA mixture was then mixed with 0.56 ml of 5% SUV of EPC and 0.83 mg of peptide (1% molar ratio of peptide to lipid) dissolved in 50 mM NaHCO.sub.3. The final mixture was shell freezed and liposomes were prepared as described in Section 2. Following hydration, a portion of the liposome solution was centrifuged at 15,000.times.g for 10 mins. at 4.degree. C., and the supernatant containing free DNA was separated from the liposome pellet. The pelleted liposomes were then washed with 2 volumes of 155 mM

NaCl and centrifuged as above. The DNA content in each fraction was determined using a .beta. counter. Percentages of DNA associated with liposome were calculated by dividing .sup.32 P counts in the washed liposome pellet by total .sup.32 P counts in the initial liposome solution. The peptide content in each fraction was determined by dissolving 100 .mu.l sample of fraction in 50 nM NaHCO.sub.3 and 5.1% (v/v) reduced triton .times.-100 (Sigma), and measuring fluorescence of the peptide (excitation at 280 nm and emission at 340 nm) using a spectrofluorimeter machine (LS 50B, Parkin, Elmer, USA). Percentages of peptide associated with liposomes were calculated by dividing peptide fluorescention level in the washed liposome pellet by peptide fluorescention level in the initial liposome solution. The results are shown in Table II. It can be seen that 48% of the DNA was associated with liposomes which co-encapsulate peptide. This result is indication that encapsulation of the peptide in the liposomes has no significant effect on encapsulation of DNA in the same liposomes.

Detailed Description Text (10):
3. In - vivo gene transfer by a liposome formulation containing factor IX expression vector

Detailed Description Text (11):
The liposome formulations were prepared as described in Sections 2 and 3. The first formulation contains 1.5 mg of human factor IX expression vector and 120 mg of EPC. The second formulation contains 1.5 mg of factor IX expression vector, 120 mg of EPC and 3.5 mg of the peptide. After the hydration, the liposomes were injected i.v. into 8 weeks old male Balb/c mice (each formulation was injected into 3 mice). At 7 days post-injection the mice were sacrificed and DNA was purified from the livers of the mice. (The liver was the organ which adsorbed most of this kind of liposome following i.v. injection).

Detailed Description Text (12):
Total DNA (50 .mu.g) was extracted from the liver of liposome-injected mice and was used to transform competent E.coli (JM109) as described in Sambrook et al. (Molecular Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1989). The transformed bacteria were plated on Luria-Bertani (medium) (LB) agar plates supplemented with 100 .mu.g Ap/ml, grown overnight at 37.degree. C. and Ap.sup.R colonies were scored. Transformation efficiency and quantitation of plasmid DNA in the liver DNA was determined using a standard curve developed by transformation of competent bacteria with 500, 100 and 10 pg of factor IX expression vector mixed with 50 .mu.g of liver DNA from untreated mice. The transformation efficiency was 5.times.10.sup.5 colonies/.mu.g of purified plasmid DNA. Because only the injected factor IX expression vector could confer Ap resistance, the number of transformed Ap resistant (Ap.sup.R) bacterial colonies was a direct indication of the plasmid DNA content within the total mouse liver DNA. As indicated in Table III, colonies of Ap.sup.R bacteria were found only in plates containing bacteria which had been transformed with DNA purified from livers of mice that had been injected with liposome-encapsulated factor IX expression plasmid. The number of Ap.sup.R bacterial colonies in plates of bacteria that were transformed with DNA purified from mice livers that were injected with the formulation containing DNA lipids and peptide was 2.2 higher than the number of Ap.sup.R bacterial colonies in plates of bacteria that were transformed with DNA purified from livers of mice that were injected with the formulation containing only lipid and DNA.

Detailed Description Text (13):
Another quantitative measurement of the amount of factor IX expression vector in total cellular DNA purified from mice livers was done by Southern blot analysis. By comparing lanes 3 and 4 in FIG. 1, it can be seen that the amount of injected plasmid DNA was 3.4 times higher in mice that were injected with the formulation containing the peptide than in mice that were injected with the formulation which does not contain the peptide. These results are in agreement with the bacteria transformation results, and indicate that the gene transfer efficiency is significantly increased when the membrane disrupting peptide is included in the liposome formulation.

Detailed Description Text (14):
4. Factor IX synthesis in mice injected with liposome formulations containing human factor IX expression vector

Detailed Description Text (15):
The liposome formulations that were described in Section 4 were injected i.v. into 2 groups of Balb/c mice (3 mice in each group). Four days post injection the mice were bled, plasma samples were prepared and tested by human factor IX specific ELISA. The plasma samples were analyzed for human FIX by ELISA using two anti-human FIX monoclonal Ab (HIX-1 and HIX-5, Sigma) as primary Ab, and rabbit anti-human FIX (Stago) as a secondary Ab. Bound rabbit Ab were detected with alkaline phosphatase conjugated anti-rabbit IgG monoclonal Ab (Sigma) and p-nitrophenyl phosphate (Sigma). Normal pooled human plasma (Stago) was used to plot a calibration curve of human FIX antigen (normal level=5 .mu.g/ml human plasma). This assay can detect as little as 10 pg human FIX in mouse plasma.

Detailed Description Text (16):
As can be seen in Table IV, significant levels of human factor IX protein were detected in the plasma of mice that had been injected with the liposome formulations containing human factor IX expression vector. The amount of human factor IX in the blood of mice that were injected with the liposome formulation containing the membrane disrupting peptide was 2.1 higher than the amount of human factor IX in the blood of mice that were injected with liposome formulation that did not contain the peptide.

Detailed Description Text (17):
5. Production of factor VIII (FVIII) in mice injected with liposome formulations containing a human factor VIII expression vector

Detailed Description Text (18):
Three liposome formulations were prepared as described in sections 2 and 3 above. The first formulation contained 1.5 mg of human factor VIII expression vector pCI-FVIII [pCI vector (Promega) containing FVIII cDNA which encodes FVIII protein with a deletion of 695 amino acids of its B domain] and 150 mg of EPC. The second formulation contained 1.5 mg of the expression vector pCI-FVIII, 150 mg of EPC and 5.2 mg of a lysosome disrupting peptide which is derived from the Influenza virus hemagglutinin protein and which increased the transfection efficiency of a complex of DNA and polylysine into cells in vitro (Plank, C., et al., J. Biol. Chem. 269:12918-12924, 1994) and has the following amino acid sequence:

Detailed Description Text (22):
The liposome formulations were injected i.v. into 6 week-old male Balb/c mice (each formulation was injected into 3 mice). As control, mice were injected with empty liposomes and a liposome-encapsulated pCI vector. At five days post-injection the mice were bled and plasma samples were prepared. Factor VIII activity in the mouse plasma was measured by a chromogenic assay (Baxter), and the level of human factor VIII in the mouse plasma was calculated as FVIII activity in the plasma of liposome-injected mice less the activity in the plasma of untreated mice.

Detailed Description Text (23):
As seen in Table V below, co-encapsulation of lysosome disrupting peptide with the expression vector in the liposomes significantly increased factor VIII production in mice. The control scrambled peptide, had no effect on factor VIII production.

Detailed Description Text (26):
(a) southern blot analysis of mouse liver DNA and transfection of liver DNA into bacteria indicated that the amount of plasmid DNA absorbed by liver cells was 2.2-3.4 times higher in mice injected with liposome encapsulated DNA and peptide than in mice injected with liposomes containing only DNA (FIG. 1, Table III).

Detailed Description Text (27):
(b) human factor IX level in the mouse plasma measured by ELISA was 2.15 times higher in mice injected with liposome encapsulated FIX expression vector and peptide than in mice injected with liposomes containing only DNA (Table IV); and

Detailed Description Text (28):
(c) the level of factor VIII in mouse plasma measured by a chromogenic assay was 2.8 times higher in mice injected with liposomes containing FVIII expression vector and

lysosome disrupting peptide than in mice injected with liposomes containing FVIII expression vector alone or co-encapsulated with control peptide (Table V).

Detailed Description Paragraph Table (2):
TABLE II Co-encapsulation of DNA and peptide in EPC liposomes Percentage of Percentage of liposome-associated liposome-associated Type of liposome formulation DNA peptide Liposomes and DNA 48 -- Liposomes, DNA and peptide 42 63

Detailed Description Paragraph Table (3):
TABLE III Bacterial transformation by total DNA purified from livers of mice injected with liposome-encapsulated plasmid DNA pg plasmid DNA/ 50 .mu.g liver DNA Injected formulation average .+-. SD (n = 3) liposomes + plasmid DNA 53 .+-. 13 liposomes + plasmid DNA + peptide 117 .+-. 31 untreated mice 0

Detailed Description Paragraph Table (4):
TABLE IV Human factor IX in mouse plasma pg hFIX/ml mouse plasma Injected formulation average .+-. SD (n = 2) Factor IX expression vector en- 45 .+-. 1.5 capsulated in liposomes Factor IX expression vector and 97 .+-. 4.5 membrane disrupting peptide encapsulated in liposomes Untreated mice 0

Detailed Description Paragraph Table (5):
TABLE V Factor VIII activity in mouse plasma 3 days post-injection of lipo- some formulations containing a factor VIII expression vector Average factor VIII activity above base ng human factor level (u/ml) VIII/ml mouse Injected formulation (n = 3) plasma.sup.(1) Untreated mice 0 0 Empty liposomes 0 0 Liposome-encapsulat- 0 0 ed control DNA Liposome encapsulated 0.38 38-76 factor VIII expression vector Liposome encapsulated 1.08 108-216 factor VIII expression vector and lysosome disrupting peptide Liposome encapsulated 0.36 36-72 factor VIII expression vector and control peptide .sup.(1) Factor VIII amounts were calculated according to 1 unit FVIII = 100-200 ng

Other Reference Publication (1):
Gregoriadis, G. , Ph.D., "Preparation of Liposome" Lipsome Technology vol. I, CRC Press, Inc., Boca Raton, FL, 1984, pp. 19-27.

Other Reference Publication (5):
Nicolau, C., et al., In Vivo Expression of Rat Insulin After Intravenous Administration of The Liposome-Entrapped Gene for Rat Insulin I, Proc. Natl. Acad. Sci. USA, vol. 80, 1983, pp. 1068-1072.

Other Reference Publication (6):
Leibiger, B., et al., Expression of Exogenous DNA in Rat Liver Vells After Liposome-Mediated Transfection In Vivo, Biochemical and Biophysical Research Communications, vol. 174, No. 3, 1991, pp. 1223-1231.

Other Reference Publication (7):
Cudd, A., et al., Intracellular Fate of Liposome-Encapsulated DNA in Mouse Liver. Analysis Using Electon Microscope Autoradiography and Subcellular Fractionation, Biochimica et Biophysica Acta, 845, 1985, pp. 477-491.

Other Reference Publication (10):
Kamata, H., et al., Amphiphilic Peptides Enhance The Efficiency of Liposome-Mediated DNA Transfection, Nucleic Acids Research, vol. 22, No. 3, 1994, pp. 536-537.

Other Reference Publication (11):
Kaneda, Y., et al., The Improved Efficient Method for Introducing Macromolecules Into Cells Using HVJ (Sendai Virus) Liposomes with Ganglioside, Experimental Cell Research, 173, 1987, pp. 56-69.

Other Reference Publication (12):
Lapidot, M., et al., Fusion-Mediated Microinjection of Liposome-Enclosed DNA Into Cultured Cells with the Aid of Influenza Virus Glycoproteins, Experimental Cell Research, 189, 1990, pp. 241-246.

Other Reference Publication (17):

Mannino, R.J., et al., Liposome Mediated Gene Transfer, Biotechniques, vol. 6, No. 7, 1988, pp. 682-690.

CLAIMS:

1. A composition comprising:

i) a polynucleic acid molecule;

ii) a liposome; and

iii) a peptide comprised of less than about 50 amino acids which disrupts membranes under acidic conditions;

said peptide and the polynucleic acid molecule being encapsulated within the liposome, wherein said peptide is derived from an influenza virus protein and said peptide comprises SEQ ID NO:4.

2. A composition comprising:

i) a polynucleic acid molecule;

ii) a liposome; and

iii) a peptide comprised of less than about 50 amino acids which disrupts membranes under acidic conditions;

said peptide and the polynucleic acid molecule being encapsulated within the liposome, wherein said peptide is derived from an influenza virus protein and said peptide comprises SEQ ID NO:1.

3. A method of introducing nucleic acid molecules to cells comprising:

(a) encapsulating a nucleic acid molecule with a liposome together with a peptide capable of disrupting membranes under acidic conditions, said peptide being comprised of less than about 50 amino acids, wherein said peptide comprises SEQ ID NO:4; and

(b) delivering said liposomes to the cells.

5. A method of introducing nucleic acid molecules to cells comprising:

(a) encapsulating a nucleic acid molecule with a liposome together with a peptide capable of disrupting membranes under acidic conditions, said peptide being comprised of less than about 50 amino acids, wherein said peptide comprises SEQ ID NO:1; and

delivering said liposomes to the cells.

WEST Search History

DATE: Monday, July 21, 2003

Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI,TDBD; PLUR=YES; OP=OR			
L2	L1 and liposome$	51	L2
L1	isotoni$ adj5 osmola$	157	L1

END OF SEARCH HISTORY